

**A novel species of *Streptomyces* isolated from Chilean Altiplano soil, *Streptomyces altiplanoense* sp. nov. and emended description of *Streptomyces chryseus* Krasil'nikov et al. 1965**

Carlos Cortes<sup>1,2</sup>, Cristina Dorador<sup>3</sup>, Peter Schumann<sup>4</sup>, Paul Herron<sup>5</sup>, Barbara Andrews<sup>2</sup>, Juan Asenjo<sup>2</sup>, Imen Nouioui<sup>1\*</sup>

1. School of Natural and Environmental Sciences, Newcastle University, Devonshire Building, Newcastle upon Tyne NE1 7RU, UK.

2. Centre for Biotechnology and Bioengineering, University of Chile, Beauchef 851, Santiago, Chile.

3. Laboratory of Microbial Complexity and Functional Ecology, Departamento de Biotecnología, Facultad de Ciencias del Mar y Recursos Biológicos & Centre for Biotechnology and Bioengineering, Universidad de Antofagasta, Chile.

4. Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Germany

5. Strathclyde Institute of Pharmacy and Biomedical Sciences, University of Strathclyde, 161 Cathedral Street, Glasgow G4 0RE, United Kingdom

**\*Corresponding author:** Imen Nouioui [imen.nouioui@newcastle.ac.uk](mailto:imen.nouioui@newcastle.ac.uk)

**Abstract**

A polyphasic approach was used for evaluating the taxonomic status of strain HST21<sup>T</sup> isolated from an extreme environment, Salar de Huasco, of Atacama Desert. The 16S rRNA gene and multi-locus sequence phylogenetic analyses assigned strain HST21<sup>T</sup> to the genus *Streptomyces* with *Streptomyces albidochromogenes* DSM 41800<sup>T</sup> and *Streptomyces flavidovirens* DSM 40150<sup>T</sup> (99.2% of rRNA gene sequence similarity) as the nearest neighbours. Digital DNA-DNA hybridization (dDDH) and average nucleotide identity (ANI) between the genome sequences of strain HST21<sup>T</sup> and its relatives, *S. albidochromogenes* DSM 41800<sup>T</sup> (35.6% and 88.2%) and *S. flavidovirens* DSM 40105<sup>T</sup> (47.2% and 88.8%), were below the thresholds of 70% and 95-96% for prokaryotic conspecific assignation. Phenotypic, chemotaxonomic and genetic results of isolate HST21<sup>T</sup> are in line with the genus *Streptomyces* and distinguish strain HST21<sup>T</sup> from its closest neighbours. Strain HST21<sup>T</sup> is characterised by the presence of LL-diaminopimelic acid in its peptidoglycan layer; glucose and ribose as cell wall sugar;

diphosphatidylglycerol (DPG), hydroxy-phosphatidylethanolamine (OH-PE), phosphatidylethanolamine (PE), phosphatidylinositol (PI), glycerophospholipids (GPL<sub>1-2</sub>), unknown lipids (L<sub>1-2</sub>) and phospholipids (PL<sub>1-2</sub>) as polar lipids; *anteiso*-C<sub>15:0</sub> (21.6%) and *anteiso*-C<sub>17:0</sub> (20.5%) as major fatty acids (>15%).

Based on these results, strain HST21<sup>T</sup> merits the recognition as novel species within the genus *Streptomyces* for which the name *Streptomyces altiplanoense* sp. nov. is proposed. The type strain is HST21<sup>T</sup> = DSM 107267<sup>T</sup> = CECT 9647<sup>T</sup>.

Members of the genus *Streptomyces* [1, 2] of the family *Streptomycetaceae* [3, 4] are well known as a preeminent source of secondary metabolites (80%) and antibiotics production [5]. This taxon encompasses Gram-positive, aerobic and heterotrophic microorganisms with extensive branched substrate and aerial mycelia [6]. Over 800 *Streptomyces* species have been validly named and characterised by the presence of LL-diaminopimelic acid (A<sub>2</sub>pm) in their peptidoglycan layer; diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannosides as major polar lipids; saturated *iso*- and *anteiso*-fatty acids as the major fatty acids; hexa- and octa-hydrogenated menaquinones with nine isoprene units as predominant isoprenologues [7, 8] and a G+C content range between 69-78 mol%. The genus *Streptomyces* is widely distributed in various ecosystems, such as soil, fresh and marine waters and clinical samples [9], in addition that they are found in extreme or poly-extreme environments, [10, 12-13].

In this context, and during our investigation of *Streptomyces* biodiversity in poly-extreme high altitude saline wetland (3800 m.a.s.l) of the Atacama Desert, *Streptomyces* strain HST21<sup>T</sup> was isolated and characterised [13,14-15] based on polyphasic taxonomic study. The isolate HST21<sup>T</sup> was found to be a new species within the evolutionary radiation of the genus *Streptomyces* for which the name *Streptomyces altiplanoense* sp. nov. is proposed.

*Streptomyces* strain HST21<sup>T</sup> was isolated from arid soil samples location (site H6) in the Salar de Huasco [14] of the Atacama Desert. The characteristic of the site H6 and the isolation procedures were carried out as described by Cortes *et al.* [13]. Isolate HST 21<sup>T</sup> was maintained on GYM (DSMZ; Medium 65) agar plates, for 7 days of incubation at 28°C, together with its closest phylogenetic relatives, *Streptomyces albidochromogenes* DSM 41800<sup>T</sup> [16], *Streptomyces flavidovirens* DSM 40150<sup>T</sup> [17-18], *Streptomyces chryseus* DSM 40420<sup>T</sup> [18-19], and *Streptomyces helveticus* DSM 40431<sup>T</sup> [18-19], which were obtained from DSMZ German

culture collection (<https://www.dsmz.de/>). All the strains were preserved in 25% v/v glycerol at -80°C.

Cultural properties of strain HST21<sup>T</sup> were examined using different agar media: International *Streptomyces* Project [ISP1-7][20] and GYM (DSMZ medium 65). A range of temperatures of 4°C, 10°C, 15°C, 25°C, 28°C, 37°C and 45°C as well as pH ranges from 6 to 11 were tested on HST21<sup>T</sup> culture using GYM media. Strain HST21<sup>T</sup> was able to grow in all the tested media but with moderate growth on ISP2. Optimal growth was detected at 28 and 37°C, pH from 6 to 10 and up to 8% (w/v) NaCl, after 7 days of incubation at 28°C. It formed a white aerial mycelium and brownish substrate mycelia with diffusible pigment after 7 days of incubation at 28°C on GYM media. More detail about the cultural characteristics of strain HST21<sup>T</sup> are listed in Table 1.

A field-emission scanning electron microscope (Tescan Vega 3 LMU: Tescan, Wellbrook Court, Girton, Cambridge, CB3 0NA) was used to describe the spore chain ornamentation and spore surface morphology of strain HST21<sup>T</sup> grown for 14 days on GYM media at 28°C. It is characterised by the presence of rectiflexible spore chains in section with spiny spore surfaces (Fig. 1) while *S. albidochromogenes* DSM 41800<sup>T</sup> and *S. flavidovirens* DSM 40150<sup>T</sup>, the nearest neighbours, have spiral and rectiflexible spore chains with smooth spore surface, respectively [7].

The ability of the studied strain HST21<sup>T</sup> to use different carbon and nitrogen sources and to grow in presence of inhibitory compounds were examined using GENIII microplates in an Omnilog device (BIOLOG Inc., Haywood, USA). The type strains of *S. albidochromogenes* and *S. flavidovirens* species were included in this test, which was carried out in duplicate. Opm package for R [21-22] version 1.06 was used to analyse the resultant data. Strain HST21<sup>T</sup> could be distinguished from its relatives cited above by its ability to metabolise rhamnose (carbon source) and quinic acid (organic acid) while strain DSM 40150<sup>T</sup> was able to oxidise D-fucose, N-acetyl-D-galactosamine, D-melibiose and methyl pyruvate (carbon sources), D-lactic acid methyl Ester, L-galactonic acid-γ-lactone, L-lactic acid (organic acids). However strain DSM 41800<sup>T</sup> could use N-acetyl-neuraminic acid (organic acid) and D-serine<sup>2</sup> and L-serine (Table 2).

Standard procedures for chemotaxonomic analyses were used to characterise strains HST21<sup>T</sup>, *S. albidochromogenes* DSM 41800<sup>T</sup> and *S. flavidovirens* DSM 40150<sup>T</sup>. Diaminopimelic acids isomers [23], cell wall sugars [24] and polar lipids profiles [25] as well as menaquinones were determined using freeze dried cells obtained following the same procedure of Cortes *et al.* [13].

The menaquinones were extracted as described by Tindall [26] and analyzed as described by Cortes *et al.* [13]. A gas chromatography (Agilent 6890 N) instrument was used to analyse the fatty acid extracts, obtained after Miller [27] and Kuykendall *et al.* [28], for all the strains cited above. The extracts were identified using the standard microbial identification (MIDI) system Version 4.5 and the ACTIN 6 database [29].

Whole cell hydrolysates of strain HST21<sup>T</sup> were rich in LL-diaminopimelic acid and, glucose, and ribose as cell wall sugars while the type strains of *S. albidochromogenes* and *S. flavidovirens* have mannose in addition. Isolate HST21<sup>T</sup> contained, in its polar lipids profile, diphosphatidylglycerol (DPG), hydroxy-phosphatidylethanolamine (OH-PE), phosphatidylethanolamine (PE), phosphatidylinositol (PI), glycerophospholipids (GPL<sub>1-2</sub>) and unknown lipids (L<sub>1-2</sub>) and phospholipids (PL<sub>1-2</sub>). The same profile was obtained for *S. flavidovirens* DSM 40150<sup>T</sup> and *S. albidochromogenes* DSM 41800<sup>T</sup> but devoid of OH-PE (Fig. S1) and with unknown aminolipids (AL<sub>1-2</sub>) and glycolipids (GL<sub>1-4</sub>) for strain DSM 41800<sup>T</sup>. Isolate HST21<sup>T</sup> has MK-9(H<sub>6</sub>) and (MK-9(H<sub>8</sub>)) as predominant menaquinone (>20%) (Table 1), like its close phylogenetic relatives; *anteiso*-C<sub>15:0</sub> (21.6%) and *anteiso*-C<sub>17:0</sub> (20.5%) were detected as the major fatty acid (>15%) of strain HST21<sup>T</sup>; however its nearest relatives *S. flavidovirens* DSM 40150<sup>T</sup> and *S. albidochromogenes* DSM 41800<sup>T</sup> had *anteiso*-C<sub>15:0</sub> (38.7%) and *anteiso*-C<sub>15:0</sub> (27.0%) and C<sub>16:0</sub> (16.0%), respectively (Table S1).

The genomic DNA extraction of strain HST21<sup>T</sup> and 16S rDNA-PCR amplification were carried out as described by Cortes *et al.* [13]. The retrieval of the nearest phylogenetic neighbours of strain HST21<sup>T</sup> was performed following the alignment of the complete 16S rRNA gene sequence (1517 bp; accession number KX130868) of isolate HST21<sup>T</sup> against those available in EzTaxon database [30].

Pairwise sequence similarities of 16S rRNA gene were estimated based on the method of Meier-Kolthoff *et al.* [31]; MUSCLE [32] software was used for multiple sequence alignments while RAXML [33] and TNT [34] were applied for Maximum-likelihood (ML) [35] and Maximum-parsimony (MP) phylogenetic trees [36], respectively. These later trees were inferred from the DSMZ phylogenomics pipeline [37] available at the Genome-to-Genome distance calculator (GGDC) web server [31] (<http://ggdc.dsmz.de/>). A rapid bootstrapping method together with the autoMRE bootstrapping criterion [38] were used for the resultant ML tree. However the resultant best topology of MP tree was obtained based on a combination of bootstrapping method of 1000 iterations with a tree-bisection-and-reconnection branch swapping method in addition to the use of ten additional random sequence replicates. The X<sup>2</sup> test of the PAUP program [39] was used to check the sequences for a compositional bias. All the trees were

rooted using the type species of the genus, *Streptomyces albus subsp. albus* NRRLB 1811<sup>T</sup> [1, 40].

Multi locus sequence phylogenetic analyses (MLSA) was carried out based on five partial housekeeping gene sequences, *atpD*, *gyrB*, *rpoB*, *recA* (recombinase A) and *trpB* [41-44]. All the genes of strain HST21<sup>T</sup> were taken from the draft genome sequence (accession number RHMC000000000) and those of the reference strains were retrieved from the ARS Microbial Genome Sequence (<http://199.133.98.43>) and the GenBank databases. A neighbour joining phylogenetic tree was constructed using the MEGA software version 7 and a Kimura 2-parameter [45] was used to estimate the genetic distance between the loci of strain HST21<sup>T</sup> and its closest phylogenetic neighbours.

Strain HST21<sup>T</sup> showed 16S rRNA gene sequence similarity values of 99.2% with *S. albidochromogenes* NBRC 101003<sup>T</sup> (12 nt of difference) and *S. flavidovirens* NBRC13039<sup>T</sup> (12 nt of difference) and 99.1% with *S. chryseus* NRRL B-12347<sup>T</sup> (13nt of difference) and *S. helveticus* NBRC 13382<sup>T</sup> (13 nt of difference). These results were reflected in the 16S rRNA tree (Fig. 2), where strain HST21<sup>T</sup> formed together with all the strains cited above a well-supported clade next to *Streptomyces hypolithicus* HSM10<sup>T</sup> [46] (Fig. 2). The phylogenetic position of *S. albidochromogenes* NBRC 101003<sup>T</sup>, *S. chryseus* NRRL B-12347<sup>T</sup>, *S. flavidovirens* NBRC13039<sup>T</sup> and *S. helveticus* NBRC 13382<sup>T</sup> (16S rRNA gene sequence similarities between 99.9% and 100%) in the same branch, which is in line with previous studies [7, 41, 47], call for taxonomic revision of the status of these species based on MLSA and genomic analyses.

In the MLSA tree, strain HST21<sup>T</sup> forms with *S. flavidovirens* DSM 40150<sup>T</sup> a subclade next to the one that encompasses the representative strains *S. albidochromogenes*, *S. chryseus* and *S. helveticus*. The latter were placed in the same branch unlike (Fig. 3). The topology of the ML and NJ MLSA trees as well as the 16S rRNA gene tree are in concordance (Fig. 2-3 and Fig. S2). These results are in coherent with the evolutionary genetic distances, which have a value of 0.0% between *S. chryseus* DSM 40420<sup>T</sup> and *S. helveticus* DSM 40431<sup>T</sup> (Table 2). However, the genetic distance between strain HST21<sup>T</sup> and the rest of its closest phylogenetic neighbours were above the threshold of 0.007 for the assigning *Streptomyces* strain to the same species [48-49] (Table 2).

The genomic DNA of strain HST21<sup>T</sup> was sequenced using Ion Torrent PGM (Personal Genome Machine) sequencer technology as described by Cortes *et al.* [13] while the *S. albidochromogenes* DSM 41800<sup>T</sup>, *S. chryseus* DSM 40420<sup>T</sup> and *S. helveticus* DSM 40431<sup>T</sup> genomes were sequenced using Illumina next-generation sequencing technology (MicrobesNG,

Birmingham, UK). The RAST server [50-51] was used for annotation of these genome sequences.

The genome sequence of strain HST21<sup>T</sup> has a size of 7.9 Mb and an *in silico* G+C content of 71.0 mol%. However, the type strain of *S. albidochromogenes* (accession number....) and *S. flavidovirens* (accession number AUBE000000000) have genome sizes of 7.4 Mb and 7.07 Mb with an *in silico* G+C content of 70.5% and 70.4%, respectively.

The GGDC server with the recommended formula 2 [31] was used to estimate the digital DNA:DNA hybridization (dDDH) between the draft genome sequence of strain HST21<sup>T</sup> and its closest phylogenetic relatives, *S. albidochromogenes* DSM 41800<sup>T</sup>, *S. chryseus* DSM 40420<sup>T</sup>, *S. flavidovirens* DSM 40105<sup>T</sup> and *S. helveticus* DSM 40431<sup>T</sup>. The OrthoANIu algorithm of the ANI Calculator [52-53] was used to calculate the average nucleotide identity (ANI) values between the strains cited above.

The obtained dDDH values between the genome of the HST21<sup>T</sup> and its closest relatives *S. albidochromogenes* DSM 41800<sup>T</sup> (35.6%), *S. chryseus* DSM 40420<sup>T</sup> (36.5%), *S. flavidovirens* DSM 40105<sup>T</sup> (47.2%), and *S. helveticus* DSM 40431<sup>T</sup> (36.0%), with which the 16S rRNA gene sequence similarities values are above 99.0%, were well below the threshold of 70% for conspecific assignation [54]. These results are in concordance with the corresponding ANI values of 88.2%, 88.4%, 88.8% and 88.2%; these results are below the cut-off point of 95-96% for delineation of prokaryotic species [55-57].

The comparison of the dDDH and ANI values estimated between the pair of the closest phylogenetic neighbours showed that only *S. chryseus* DSM 40420<sup>T</sup> and *S. helveticus* DSM40431<sup>T</sup> have dDDH (95.3%) and ANI (99.4%) values above the described threshold of 70% and 96%, respectively. These results are coherent with their phylogenetic position in the 16S rRNA gene and MLSA tree and also with their phenotypic and chemotaxonomic features. Both strains have DPG, PE, PI, GPL as major polar lipids and *anteiso* C<sub>15:0</sub> (19.9%) and *iso* C<sub>16:0</sub> (19.3%) as the major fatty acids (>15%). In light of these findings, it is proposed that *S. helveticus* species be recognised as heterotypic synonym of *S. chryseus*. Therefore, an emended description of this later species is necessary.

In conclusion, strain HST21<sup>T</sup> showed phenotypic, genetic and genomic data distinct from its closest phylogenetic relatives and consequently, it merits the recognition as a new species, namely as *Streptomyces altiplanoense* sp. nov.

## **Description of *Streptomyces altiplanoense* sp. nov.**

*Streptomyces altiplanoense* (al.ti.pla.no.en.se N.L. masc. n. *altiplanoense* referring to the site of Chilean Altiplano where the strain was isolated)

Aerobic, Gram-positive actinobacteria produce white aerial mycelium and brownish substrate mycelia with diffusible pigment were observed after 7 days of incubation at 28°C on GYM media. It has rectiflexible spore chains in section with spiny spore surfaces. Optimal growth of strain HST21<sup>T</sup> on GYM agar medium at 28°C. Strain HST21<sup>T</sup> was able to metabolise sucrose, stachyose, D-raffinose, D-fructose, D-galactose (carbon sources); L-pyroglutamic acid, quinic acid,  $\beta$ -hydroxy-butyric acid,  $\alpha$ -keto-butyric acid, butyric acid (organic acids); L-arginine (amino acid); and grow in presence of aztreonam, lithium chloride and Tween 40 (inhibitory compounds) and sodium bromate, 1% sodium lactate (salts) (Table 1). Whole cell hydrolysates of strain HST21<sup>T</sup> were rich in LL-diaminopimelic acid in its peptidoglycan and, glucose, and ribose in its cell wall sugars. It is characterised by the presence of diphosphatidylglycerol (DPG), hydroxy- phosphatidylethanolamine (OH-PE), phosphatidylethanolamine (PE), phosphatidylinositol (PI), glycopospholipids (GPL<sub>1-2</sub>) and unknown lipids (L<sub>1-2</sub>) and phospholipids (PL<sub>1-2</sub>) as polar lipids; *anteiso*-C<sub>15:0</sub> (21.6%) and *anteiso*-C<sub>17:0</sub> (20.5%) as major fatty acids (>15%). The menaquinone profile contained MK-9(H<sub>6</sub>) (6%, MK-9(H<sub>8</sub>) 25%, MK-9(H<sub>4</sub>) 8%, MK-7(H<sub>2</sub>) 8%, MK-8(H<sub>2</sub>) 6%, MK-9(H<sub>2</sub>) 3%, MK-10 2%.

The genome size is 7.9 Mb with an *in silico* G+C content of 71.0%. The type strain HST21<sup>T</sup> (DSM 107267<sup>T</sup> = CECT 9647<sup>T</sup>) was isolated from hyper arid soil of Salar de Huasco in the Atacama Desert, Chile.

## **Amended description of *Streptomyces chryseus* (Krasil'nikov *et al.* 1965; Pridham 1970)**

The description is as given by Kämpfer (2012) with following modification and additions after inclusion of *S. helveticus*. Spore chains in section *Retinaculiaperti* to *Spirales* but rectiflexible spore chains may also be common; spore surface is smooth. Fatty acids profile (>5%) contains. *anteiso*-C<sub>15:0</sub> (19.9%), *anteiso* C<sub>17:0</sub> (12.5%), *iso*-C<sub>16:0</sub> (19.3%), *iso* C<sub>15:0</sub> (8.6%), C<sub>16:1</sub> *cis* 9 (6.9%), C<sub>16:0</sub> (5.7%) and C<sub>16:0</sub> methyl 9 (5.9%). Polar lipid pattern has DPG, PE, PI, GPL, AL, L<sub>1-2</sub> and PL. Genome size is 7.1 -7.6 Mb with an *in silico* G+C content of 71.2-71.3%.

The type strain is AS 4.1694, ATCC 19829, CBS 678.72, DSM 40420, NBRC 13377, JCM 4737, NCIMB 10041, NRRL B-12347, NRRL-ISP 5420, RIA 1338, VKM Ac-200.

## **Acknowledgements**

This project was supported by the School of Natural and Environmental Sciences at Newcastle University. IN is grateful to Newcastle University for a postdoctoral fellowship. CC is grateful to CONICYT for PFCHA/DOCTORADO BECAS CHILE/2016 – 21160585 fellowship and operational expenses. This work was also financially supported by the Basal Centres Programme of CONICYT (Chile) for funding the Centre for Biotechnology and Bioengineering, CeBiB (project FB0001) and FONDECYT 1110953; 11181773 grants.

## Conflicts of interest

The authors declare that they have no conflicts of interest.

## References

1. **Waksman SA, Henrici AT.** The nomenclature and classification of the actinomycetes. *J Bacteriol* 1945;46:4.
2. **Witt D, Stackebrandt E.** Unification of the Genera *Streptoverticillum* and *Streptomyces*, and amendment of *Streptomyces* Waksman and Henrici 1943, 339AL. *Syst Appl Microbiol* 1990;13:361–371.
3. **Kim SB, Lonsdale J, Seong CN, Goodfellow M.** *Streptacidiphilus* gen. nov., acidophilic actinomycetes with wall chemotype I and emendation of the family *Streptomycetaceae* (Waksman and Henrici (1943)AL) emend. Rainey et al. 1997. *Antonie van Leeuwenhoek* 2003;83:107–116.
4. **Zhi XY, Li WJ, Stackebrandt E.** An update of the structure and 16S rRNA gene sequence-based definition of higher ranks of the class *Actinobacteria*, with the proposal of two new suborders and four new families and emended descriptions of the existing higher taxa. *Int J Syst Evol Microbiol* 2009;59:589–608.
5. **Watve M, Tickoo R, Jog M, Bhole B.** How many antibiotics are produced by the genus *Streptomyces* ? *Arch Microbiol* 2001;176:386–390.
6. **Angert ER.** Alternatives to binary fission in bacteria. *Nat Rev Microbiol* 2005;3:214–224.
7. **Kämpfer P.** Genus I. *Streptomyces*. In: Whitman, W., Goodfellow, M., Kämpfer, P., Busse, H.-J., Trujillo, M., Ludwig, W., Suzuki, K.-i., Parte A (Eds). *Bergey's Manual of Systematic Bacteriology*, vol 5, 2<sup>nd</sup> edn. The *Actinobacteria*, Part B. New York: Springer; 2012. pp. 1455–1767.
8. **Kroppenstedt R.** Fatty acid and menaquinone analysis of actinomycetes and related organisms. In: Goodfellow, M., Minnikin, D. E (Eds). *Chemical Methods in Bacterial Systematics*. London:



Elsevier Science & Technology Books; 1985. pp. 173–199.

9. **Kapadia M, Rolston KVI, Han XY.** Invasive *Streptomyces* infections: Six cases and literature review. *Am J Clin Pathol* 2007;127:619-624.
10. **Hong K, Gao A-H, Xie Q-Y, Gao HG, Zhuang L, et al.** Actinomycetes for Marine Drug Discovery Isolated from Mangrove Soils and Plants in China. *Mar Drugs* 2009;7:24–44.
11. **Tiwari K, Gupta RK.** Rare actinomycetes: A potential storehouse for novel antibiotics. *Crit Rev Biotechnol* 2012;32:108-132.
12. **Guo X, Liu N, Li X, Ding Y, Shang F, et al.** Red soils harbor diverse culturable actinomycetes that are promising sources of novel secondary metabolites. *Appl Environ Microbiol* 2015;81:3086-3103.
14. **Cortes C.** Isolation of *Streptomyces* strains producers of bioactive compounds from Salar de Huasco, Atacama Desert (Thesis). University of Antofagasta 2013.
13. **Cortes C, Dorador C, Schumann P, Andrews B, Asenjo J, Nouioui I.** *Streptomyces huascoense* sp. nov., an haloalkalotolerant actinobacteria isolated from a high altitude saline wetland at the Chilean Altiplano. *Int J Syst Evol Microbiol* 2019. Accepted
15. **Dorador C, Meneses D, Urtuvia V, Demergasso C, Vila I, et al.** Diversity of bacteroidetes in high-altitude saline evaporitic basins in northern Chile. *J Geophys Res Biogeosciences* 2009;114:1–11.
16. **Gause GF, Preobrazhenskaya TP, Sveshnikova MA, Terekhova LP, Maximova TS.** A guide for the determination of actinomycetes. Genera *Streptomyces*, *Streptoverticillium*, and *Chainia*. Moscow: URSS, Nauka. 1983.
17. **Kudrina ES.** In: Gauze, G.F., Preobrazhenskaya, T.P., Kudrina, E.S., Blinov, N.O., Ryabova I.D., Sveshnikova, M.A. (Eds). Problems of classification of actinomycetes-antagonists. Government Moscow: Publishing house of medical literature, Medgiz; 1957. pp. 1-398.
18. **Pridham TG, Hesseltine CW And Benedict RG.** A guide for the classification of streptomycetes according to selected groups: placement of strains in morphological sections. *Applied Microbiology*, 1958, 6, 52-79.
19. **Krasil'nikov NA, Korenyako AI, Nikitina NI.** Actinomycetes of the yellow group. In: Krasil'nikov, N.A. (ed.). Biology of selected groups of Actinomycetes (in Russian). Institute of Microbiology, Academy of Science. Moscow: Publishing Firm Nauka; 1965. pp. 1-372.
20. **Shirling EB, Gottlieb D.** Methods for characterization of *Streptomyces* species. *Int J Syst Bacteriol* 1966;16:313–340.
21. **Vaas LAI, Sikorski J, Michael V, Göker M, Klenk HP.** Visualization and curve-parameter estimation strategies for efficient exploration of phenotype microarray kinetics. *PLoS One* 2012;7:e34846.
22. **Vaas LAI, Sikorski J, Hofner B, Fiebig A, Buddruhs N, et al.** Opm: An R package for analysing OmniLog®phenotype microarray data. *Bioinformatics* 2013;29:1823-1824..

- 305 23. **Staneck JL, Roberts GD.** Simplified Approach to Identification of Aerobic Actinomycetes by  
306 Thin-Layer Chromatography. *Appl Microbiol* 1974;28:226-231.
- 307 24. **Lechevalier MP, Lechevalier H.** Chemical composition as a criterion in the classification of  
308 aerobic actinomycetes. *Int J Syst Bacteriol* 1970;20:435–443.
- 309 25. **Minnikin DE, O'Donnell AG, Goodfellow M, Alderson G, Athalye M, et al.** An integrated  
310 procedure for the extraction of bacterial isoprenoid quinones and polar lipids. *J Microbiol*  
311 *Methods* 1984;2:233-241.
- 312 26. **Tindall BJ.** Lipid composition of *Halobacterium lacusprofundi*. *FEMS Microbiol Lett*  
313 1990;66:199–202.
- 314 27. **Miller LT.** Single derivatization method for routine analysis of bacterial whole-cell fatty acid  
315 methyl esters, including hydroxy acids. *J Clin Microbiol* 1982;16:584-586.
- 316 28. **Kuykendall LD, Roy MA, O'Neill JJ, Devine TE.** Fatty Acids, antibiotic resistance, and  
317 deoxyribonucleic acid homology groups of *Bradyrhizobium japonicum*. *Int J Syst Evol Microbiol*  
318 1988;38:358:361.
- 319 29. **Sasser M.** Identification of bacteria by gas chromatography of cellular fatty acids. 1990  
320 Technical Note 101.
- 321 30. **Kim OS, Cho YJ, Lee K, Yoon SH, Kim M, et al.** Introducing EzTaxon-e: A prokaryotic 16s  
322 rRNA gene sequence database with phylotypes that represent uncultured species. *Int J Syst Evol*  
323 *Microbiol* 2012;62:716–721.
- 324 31. **Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M.** Genome sequence-based species  
325 delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics*  
326 2013;14:60.
- 327 32. **Edgar RC.** MUSCLE: multiple sequence alignment with high accuracy and high throughput.  
328 *Nucleic Acids Res* 2004;32:1792–1797.
- 329 33. **Stamatakis A.** RAxML version 8: A tool for phylogenetic analysis and post-analysis of large  
330 phylogenies. *Bioinformatics* 2014;30:1312-1213.
- 331 34. **Goloboff PA, Farris JS, Nixon KC.** TNT, a free program for phylogenetic analysis. *Cladistics*  
332 2008;24:1-13.
- 333 35. **Felsenstein J.** Evolutionary trees from DNA sequences: a maximum likelihood approach. *J Mol*  
334 *Evol* 1981;17:368–376.
- 335 36. **Fitch WM.** Toward defining the course of evolution: Minimum change for a specific tree  
336 topology. *Syst Biol* 1971;20:406–416.
- 337 37. **Meier-Kolthoff JP, Hahnke RL, Petersen J, Scheuner C, Michael V, et al.** Complete genome  
338 sequence of DSM 30083<sup>T</sup>, the type strain (U5/41T) of *Escherichia coli*, and a proposal for  
339 delineating subspecies in microbial taxonomy. *Stand Genomic Sci* 2014;9:2.
- 340 38. **Pattengale ND, Alipour M, Bininda-Emonds ORP, Moret BME, Stamatakis A.** How many  
341 bootstrap replicates are necessary? *J Comput Biol* 2010;17:337-354.

39. **Swofford DL.** PAUP\*: Phylogenetic Analysis Using Parsimony (\*and Other Methods), Version 4.0. Sunderland: Sinauer Associates; 2002.
40. **Doria RT.** Su di alcune specie di "*Streptothrix*" trovate nell'aria studate in rapporto a quelle già note a specialmente all' "*Actinomyces*". *Annali dell'Istituto d'Igiene Sperimentale, Università Roma*, 1891;1:399-438.
41. **Labeda DP, Dunlap CA, Rong X, Huang Y, Doroghazi JR, et al.** Phylogenetic relationships in the family *Streptomycetaceae* using multi-locus sequence analysis. *Antonie Van Leeuwenhoek* 2017;110:563–583.
42. **Busarakam K, Bull AT, Girard G, Labeda DP, Van Wezel GP, et al.** *Streptomyces leeuwenhoekii* sp. nov., the producer of chaxalactins and chaxamycins, forms a distinct branch in *Streptomyces* gene trees. *Antonie van Leeuwenhoek* 2014;105:849–861.
43. **Idris H, Labeda DP, Nouioui I, Castro JF, Montero-Calasanz MDC, et al.** *Streptomyces aridus* sp. nov., isolated from a high altitude Atacama Desert soil and emended description of *Streptomyces noboritoensis* Isono et al. 1957. *Antonie van Leeuwenhoek* 2017;110:705-717.
44. **Labeda DP.** Taxonomic evaluation of putative *Streptomyces scabiei* strains held in the ARS Culture Collection (NRRL) using multi-locus sequence analysis. *Antonie van Leeuwenhoek, Int J Gen Mol Microbiol* 2016;109:349–356.
45. **Kimura M.** A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 1980; 16:111-120.
46. **Le Roes-Hill M, Rohland J, Meyers PR, Cowan DA, Burton SG.** *Streptomyces hypolithicus* sp. nov., isolated from an Antarctic hypolith community. *Int J Syst Evol Microbiol* 2009;**59**, 2032-2035.
47. **Nouioui I, Carro L, García-López M, Meier-Kolthoff JP, Woyke T, et al.** Genome-based taxonomic classification of the phylum *Actinobacteria*. *Front Microbiol* 2018. DOI: 10.3389/fmicb.2018.02007.
48. **Rong X, Huang Y.** Multi-locus sequence analysis. Taking prokaryotic systematics to the next level. *Methods Microbiol* 2014;41:221-251.
49. **Rong X, Huang Y.** Taxonomic evaluation of the *Streptomyces hygroscopicus* clade using multilocus sequence analysis and DNA-DNA hybridization, validating the MLSA scheme for systematics of the whole genus. *Syst Appl Microbiol* 2012;35:7–18.
50. **Aziz RK, Bartels D, Best A, DeJongh M, Disz T, et al.** The RAST Server: Rapid annotations using subsystems technology. *BMC Genomics* 2008;9:75.
51. **Overbeek R, Olson R, Pusch GD, Olsen GJ, Davis JJ, et al.** The SEED and the Rapid Annotation of microbial genomes using Subsystems Technology (RAST). *Nucleic Acids Res* 2014;42:D206-D214.
52. **Yoon SH, Ha S min, Lim J, Kwon S, Chun J.** A large-scale evaluation of algorithms to calculate average nucleotide identity. *Antonie van Leeuwenhoek* 2017;110:1281-1286.

53. **Lee I, Kim YO, Park SC, Chun J.** OrthoANI: An improved algorithm and software for calculating average nucleotide identity. *Int J Syst Evol Microbiol* 2016;66:1100-1103.
54. **Wayne LG, Brenner DJ, Colwell RR, Grimont PAD, Kandler O, *et al.*** Report of the Ad Hoc comitee on reconciliation of approaches to bacterial systematics. *Int J Syst Bacteriol* 1987;37:463-464.
55. **Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, *et al.*** DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. *Int J Syst Evol Microbiol* 2007;57:81-91.
56. **Richter M, Rosselló-Móra R.** Shifting the genomic gold standard for the prokaryotic species definition. *Proc Nat Acad Sci* 2009;106:19126-19131.
57. **Chun J, Rainey FA.** Integrating genomics into the taxonomy and systematics of the Bacteria and Archaea. *Int J Syst Evol Microbiol* 2014; 64:316-324.

### Figure legends

**Fig. 1.** Scanning electron micrograph of strain HST21<sup>T</sup> showing “rectiflexible” spore chains section and spores with a spiny surface, after 14 days of incubation on GYM agar plates at 28°C.

**Fig. 2.** Maximum-likelihood phylogenetic tree based on almost complete 16S rRNA gene sequences constructed using the GTR+GAMMA model showing the phylogenetic relationship between isolate HST21<sup>T</sup> and its relative within the genus *Streptomyces*. The numbers above the branches are bootstrap support values greater than 60% for ML (left) and MP (right).

**Fig. 3.** Maximum-likelihood phylogenetic tree based on concatenated sequences of five genes, *atpD*, *gyrB*, *recA*, *rpoB* and *trpB*, showing the phylogenetic relationship between isolate HST21<sup>T</sup> and its relatives within the genus *Streptomyces*. The numbers above the branches are bootstrap support values greater than 60% for ML (left) and MP (right).

**Table 1.** Growth and cultural features of strain HST21<sup>T</sup> after 7 days of incubation at 28°C

Medium	Growth	Substrate mycelium colour	Aerial mycelium colour	Diffusible pigments
Tryptone-yeast extract agar (ISP1)	++	Deep reddish brown	Dark brown	Strong reddish brown
Yeast extract-malt extract agar (ISP 2)	+	White	White	-

Oatmeal agar (ISP 3)	+++	-	White	Deep reddish brown
Inorganic salts-starch agar (ISP 4)	++	Dark grayish yellow	Light grayish olive	Vivid greenish yellow
Glycerol-asparagine agar (ISP 5)	++	Light olive brown	Strong yellowish brown	Strong yellow
Tyrosine agar (ISP 7)	++	Dark yellowish brown	Light olive brown	Dark Yellow
GYM (DSMZ 65)	+++	Strong brown	White	Deep reddish brown

410

411

412

413

414

415

**Table 2.** Phenotypic features that distinguish strain HST21<sup>T</sup> from its nearest phylogenetic neighbours *S. albidochromogenes* DSM 41800<sup>T</sup> and *S. flavidovirens* DSM 40150<sup>T</sup>

	Strain HST21 <sup>T</sup>	<i>S. albidochromogenes</i> DSM 41800 <sup>T</sup>	<i>S. flavidovirens</i> DSM 40150 <sup>T</sup>
<b>Carbon utilization</b>			
D-arabitol ,D-fructose, $\alpha$ -D-lactose, myo-inositol, D-mannitol, D-raffinose, pectin, stachyose and turanose	+	-	+
D-fucose , N-Acetyl-D-galactosamine and D-melibiose	-	-	+
L-rhamnose	+	-	-
Methyl pyruvate	-	-	+
<b>Aminoacids</b>			
D-Serine 2, L-Serine	-	+	-
Glycine-proline	+	+	-
<b>Organic Acids</b>			
Bromo-succinic acid, citric acid and l-pyroglutamic acid	+	-	+
D-lactic acid methyl ester, L-galactonic acid- $\gamma$ -lactone and L-lactic acid	-	-	+
D-malic acid and sodium formate	+	+	-
N-acetyl-neuraminic acid	-	+	-
Quinic acid	+	-	-
<b>Inhibitory compounds</b>			
Rifamycin sv, 1% sodium lactate	+	+	-
8% NaCl	+	-	-
Menaquinone patterns	MK-9(H <sub>6</sub> ) 26%, MK-9(H <sub>8</sub> ) 25%, MK-9(H <sub>4</sub> ) 8%, MK-7(H <sub>2</sub> ) 8%, MK-8(H <sub>2</sub> ) 6%, MK-9(H <sub>2</sub> ) 3%, MK-10 2%	MK-9(H <sub>8</sub> ) 76%, MK-9(H <sub>6</sub> ) 7%, MK-9(H <sub>4</sub> ) 7%, MK-9(H <sub>2</sub> ) 6%, MK- 10(H <sub>2</sub> ) 1%	MK-9(H <sub>8</sub> ) 52%, MK-9(H <sub>6</sub> ) 22%, MK-9(H <sub>4</sub> ) 11%, MK-9(H <sub>2</sub> ) 9%, MK-10(H <sub>2</sub> ) 3%, MK-8(H <sub>4</sub> ) 2%, MK-9 1%

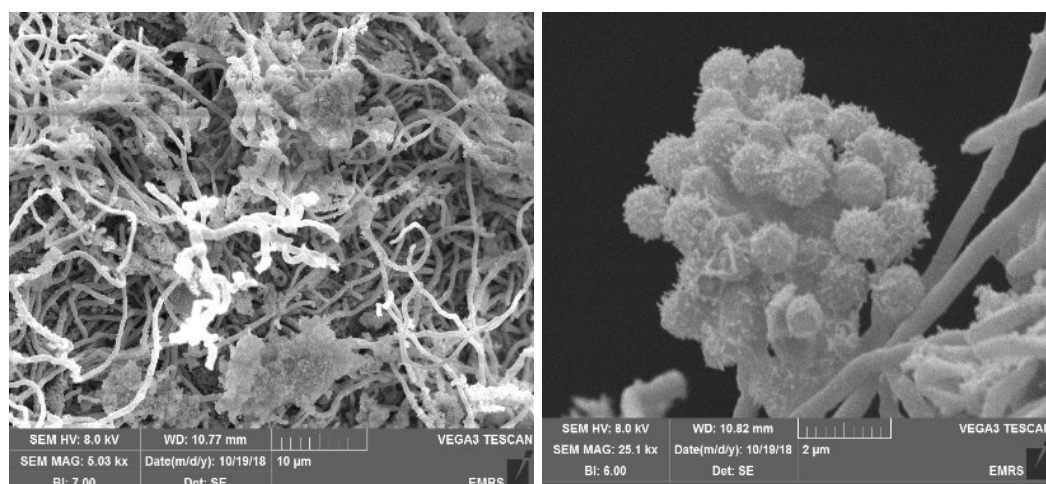
+ positive reaction; - negative reaction. All the strains were able to metabolise Dextrin, D-Maltose, L-Fucose, D-Trehalose, N-Acetyl- $\beta$ -D-Mannosamine, D-Cellobiose,  $\beta$ -Gentobiose,  $\beta$ -Methyl-D-Glucoside, D-Salicin, N-Acetyl-D-Glucosamine, D-Glucose, D-Mannose, D-Galactose, Inosine, Glycerol, D-Glucose-6-Phosphate, D-Fructose-6-Phosphate, Gelatin Sucrose and Tween 40 (carbon source); L-Arginine, L-Alanine, L-Aspartic Acid, L-Glutamic Acid and L-Histidine (aminoacids); Butyric Acid,  $\beta$ -Hydroxy-Butyric Acid, D-Gluconic Acid,  $\alpha$ -Keto Glutaric Acid, L-Malic Acid,  $\gamma$ -Amino-n-Butyric Acid,  $\alpha$ -hydroxy-Butyric Acid,  $\alpha$ -Keto-Butyric Acid, Acetoacetic Acid, Propionic Acid and Acetic Acid (organic acids); to grow in presence of Nalidixic Acid, Lithium Chloride, Potassium Tellurite, Aztreonam and Sodium Bromate (inhibitory compounds); and at 1-4% (w/v) NaCl and pH 6-8. In contrast none of the strains used D-Sorbitol, 3-O-Methyl-D-Glucose and Glucuronamide (carbon source); D-Aspartic Acid and D-Serine #1 (aminoacids); D-Glucuronic Acid, D-Galacturonic Acid, p-Hydroxy-Phenylacetic Acid, D-Saccharic Acid and Mucic Acid (organic acids); and were unable to grow in presence of Guanidine Hydrochloride, Tetrazolium Blue and Violet, Troleandomycin, Vancomycin, Minocycline, Lincomycin, Niaproof, Fusidic Acid and pH 5.

**Table 3.** Evolutionary distances between strain HST21<sup>T</sup> and its phylogenetic relatives based on the concatenated partial sequences of the five housekeeping genes: *atpD*, *gyrB*, *recA*, *rpoB* and *trpB* using Kimura 2-parameter.

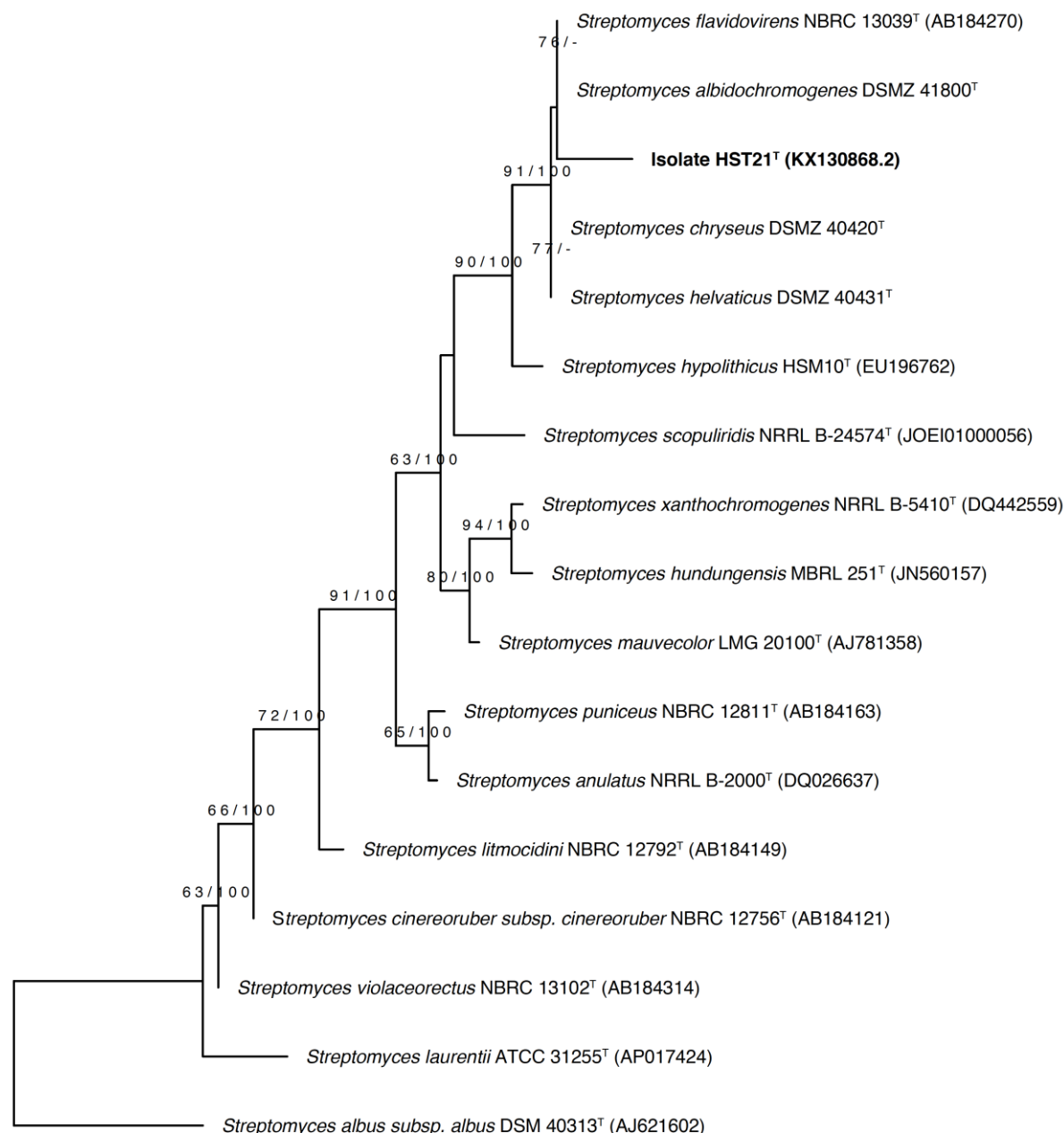
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	Strain HST21 <sup>T</sup>	-														
2	<i>Streptomyces violaceorectus</i> NRRL B-12181 <sup>T</sup>	0.104	-													
3	<i>Streptomyces xanthochromogenes</i> NRRL B-5410 <sup>T</sup>	0.110	0.103	-												
4	<i>Streptomyces anulatus</i> NRRL B-2000 <sup>T</sup>	0.113	0.107	0.109	-											
5	<i>Streptomyces_mauvecolor</i> NRRL B-24302 <sup>T</sup>	0.102	0.101	0.046	0.103	-										
6	<i>Streptomyces hypolithicus</i> NRRL B-24669 <sup>T</sup>	0.094	0.121	0.106	0.132	0.101	-									
7	<i>Streptomyces litmocidini</i> NRRL B-3635 <sup>T</sup>	0.104	0.063	0.107	0.108	0.102	0.120	-								
8	<i>Streptomyces puniceus</i> NRRL ISP-5083 <sup>T</sup>	0.115	0.114	0.118	0.132	0.108	0.135	0.109	-							
9	<i>Streptomyces albidochromogenes</i> DSM 41800 <sup>T</sup>	0.063	0.107	0.117	0.103	0.109	0.118	0.107	0.104	-						
10	<i>Streptomyces albus</i> subsp. <i>albus</i> NRRL B-1811 <sup>T</sup>	0.143	0.136	0.145	0.129	0.135	0.148	0.143	0.136	0.130	-					
11	<i>Streptomyces flavidovirens</i> DSM 40150 <sup>T</sup>	0.059	0.112	0.119	0.101	0.113	0.111	0.111	0.106	0.052	0.131	-				
12	<i>Streptomyces helveticus</i> DSM 40431 <sup>T</sup>	0.085	0.121	0.119	0.113	0.115	0.116	0.118	0.118	0.041	0.135	0.068	-			
13	<i>Streptomyces hundingensis</i> BH38	0.105	0.101	0.044	0.104	0.038	0.108	0.103	0.106	0.114	0.140	0.120	0.120	-		
14	<i>Streptomyces laurentii</i> ATCC 31255 <sup>T</sup>	0.112	0.073	0.101	0.100	0.101	0.120	0.069	0.101	0.110	0.138	0.111	0.114	0.102	-	



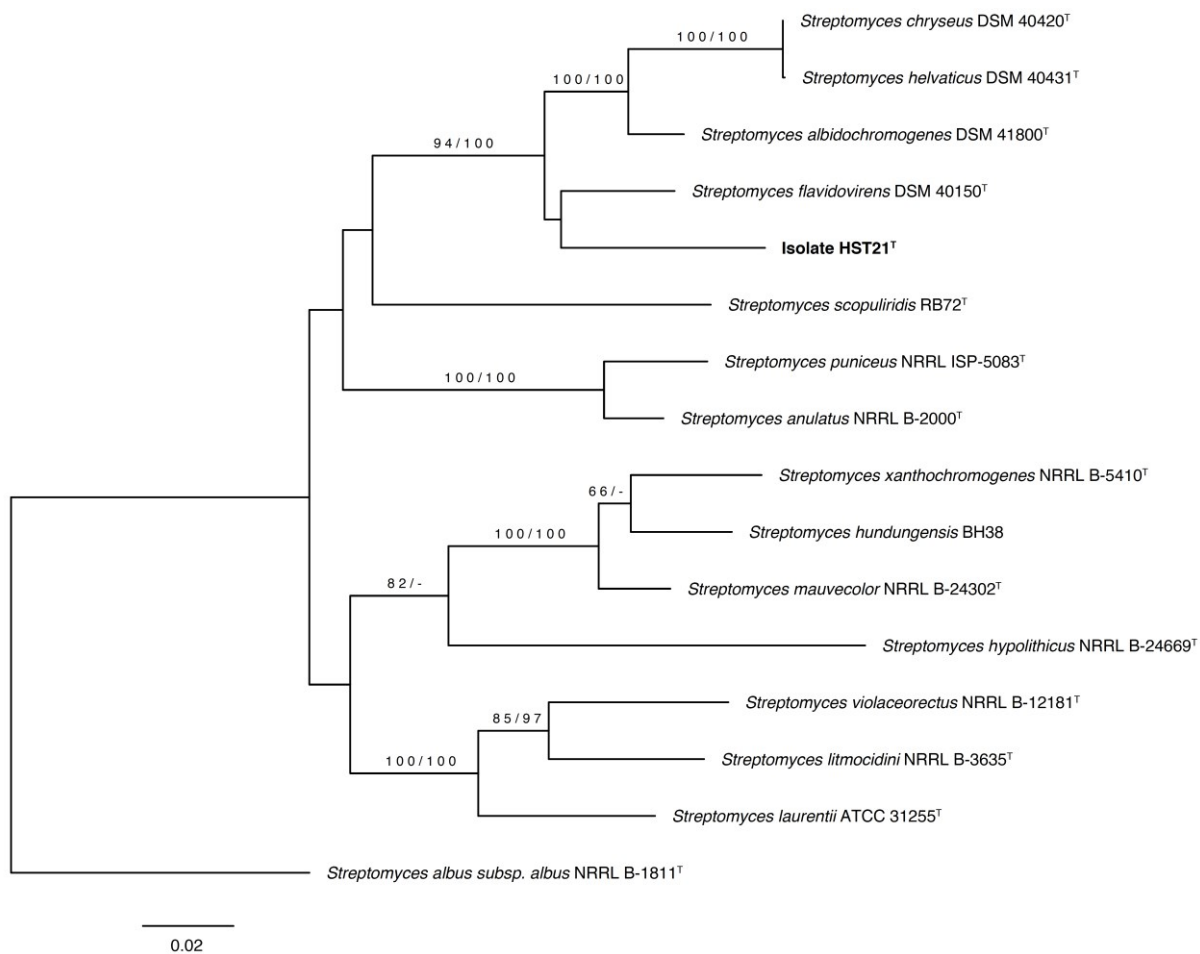
1	<i>Streptomyces</i>															
5	<i>scopuliridis</i>	0.115	0.1	0.1	0.1	0.0	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.1	0.1	-
	RB72 <sup>T</sup>		14	08	03	98	15	17	15	05	36	98	11	10	17	
1	<i>Streptomyces</i>															
6	<i>chryseus</i>	0.085	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.0	0.1	0.0	0.0	0.1	0.1	0.1
	DSM 40420 <sup>T</sup>		21	18	12	14	15	18	17	40	34	67	00	19	14	10



**Fig. 1.** Scanning electron micrograph of strain HST21<sup>T</sup> showing “rectiflexible” spore chains section and spores with a spiny surface, after 14 days of incubation on GYM agar plates at 28°C.



**Fig. 2.** Maximum-likelihood phylogenetic tree based on almost complete 16S rRNA gene sequences constructed using the GTR+GAMMA model showing the phylogenetic relationship between isolate HST21<sup>T</sup> and its relative within the genus *Streptomyces*. The tree was inferred using the GTR+GAMMA model and rooted using the 16S rRNA sequence of *Streptomyces albus subsp. albus* DSM 40313<sup>T</sup>. The numbers above the branches are bootstrap support values greater than 60% for ML (left) and MP (right).



**Fig. 3.** Maximum-likelihood phylogenetic tree based on concatenated sequences of five genes showing the phylogenetic relationship between isolate HST21<sup>T</sup> and its relatives within the genus *Streptomyces*. The tree was inferred using the GTR+GAMMA model and rooted with *S. albus subsp. albus* NRRL B-1811<sup>T</sup> as outgroup. The numbers above the branches are bootstrap support values greater than 60% for ML (left) and MP (right).